

Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 45826704

Data Requirement:


PMRA DATA CODE	{.....}
EPA DP Barcode	D290348
OECD Data Point	
EPA MRID	45826704
EPA Guideline	72-1(a)

Test material: BAS 500 00F (Headline Fungicide)**Purity:** 247.83 g/L a.i.**Common name:** Pyraclostrobin**Chemical name:** IUPAC: Not reported

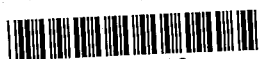
CAS name: Not reported

CAS No.: Not reported

Synonyms: Not reported

Primary Reviewer: Rebecca Bryan
Staff Scientist, Dynamac Corporation**Signature:**
Date: 3/1/04**QC Reviewer:** Gregory S. Hess
Staff Scientist, Dynamac Corporation**Signature:**
Date: 3/1/04**Approved By:** Lewis Ross Brown, Biologist
OPP/EFED/ERB**Date:** 02/10/05{ **Reference/Submission No.:****Company Code:****Active Code:****EPA PC Code:** 099100**Date Evaluation Completed:**

CITATION: Zok, S. 1999. BAS 500 00F (Headline Fungicide)- Acute Toxicity Study on the Bluegill (*Lepomis macrochirus* RAF.) in a Static System (96 hours). Unpublished study performed by Department of Toxicology, BASF Aktiengesellschaft, Ludwigshafen, Germany. Laboratory Project Identification No. 14F0185/975118 (BASF Reg. Doc. No. 1999/11836). Study sponsored by BASF-Aktiengesellschaft; D-67056 Ludwigshafen, Federal Republic of Germany. Study submitted by BASF Corporation, Agricultural Products, Research Triangle Park, NC. Study initiated August 30, 1999 and completed September 3, 1999. Final report issued November 30, 1999.



2022946

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EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, Bluegill sunfish (*Lepomis macrochirus*) were exposed to BAS 500 00F (Headline Fungicide, a.i. Pyraclostrobin) at nominal concentrations of 0.0 (negative control), 0.0032, 0.0058, 0.010, 0.018, 0.032, and 0.058 ppm under static conditions. Mean-measured concentrations (1 plus 96 hours) were < LOQ (control), 0.0028, 0.0046, 0.0080, 0.0146, and 0.0299 ppm. A mean measured value for the nominal 0.058 ppm treatment group was not reported.

After 96 hours of exposure, 10, 100, and 100% mortality was observed in the nominal 0.018, 0.032, and 0.058 ppm treatment groups, respectively. No mortalities were observed in the control, 0.0032, 0.0058, or 0.010 ppm treatment groups. The sub-lethal effects included a narcotic-like state in the nominal 0.058 ppm (two fish), 0.032 ppm (one fish) and apathy in the 0.018 ppm (one fish) treatment groups at 1, 24 and 48 hours, respectively.

The 96 hour recovery for the measured 0.0021(65.6%), 0.0036 (62.1%), 0.0063 (63.0%) and 0.0106 ppm (58.9%) treatment groups was less than 70% of the nominal 0.0032, 0.0058, 0.010, and 0.018 ppm treatment groups, respectively, and the 0.058 ppm nominal test concentration was not measured at 96 hours. Consequently, this study is classified as **Invalid**. This study is not scientifically sound because the actual concentrations which fish were exposed to are unknown. The test material declined to an unacceptable level, reportedly because the test compound shows a high affinity to glass surfaces, and it may have adhered to the test chambers (p. 44). The study author provided no further details regarding the poor solubility, nor made mention that attempts were made to improve test material recovery after 96 hours. This study does not satisfy the guideline requirement for an acute toxicity study with freshwater fish (Subdivision E, §72-1).

Results Synopsis

Test Organism Size/Age (mean Weight or Length): 3.79 g; 7.03 cm
Test Type (Flow-through, Static, Static Renewal): Static

96-Hour; Invalid study-results not reported

LC₅₀: 95% C.I.:
NOAEC:
LOAEC:
Endpoints affected:
Most sensitive endpoint:

I. MATERIALS AND METHODS

GUIDELINES FOLLOWED: The study protocol was based on procedures outlined in the OECD guidelines for Testing of Chemicals No. 203: "Fish Acute Toxicity Test" (1984) and EEC Directive 84/449, Methods for the determination of ecotoxicity, Publication No. L251, C-1: "Acute Toxicity for Fish" (1984). Deviations from U.S. EPA guidelines §72-1 included:

1. The dilution water hardness (250 mg CaCO₃/L) was five times higher than recommended (40-48 mg CaCO₃/L).

Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 45826704

2. Test specimen age at test initiation was not reported.
3. The test temperature was 23-24°C (continuous measurements: 23.9-25.8°C), which is slightly above the US EPA recommended 22°C ± 1.
4. The test pH, 8.2-8.5, was higher than the US EPA recommended 7.2-7.6.
5. Treatment group nominal concentrations were spaced by a factor of 1.8 rather than the US. EPA. recommended 1.5.
6. The 96 hour recovery for the measured 0.0021(65.6%), 0.0036 (62.1%), 0.0063 (63.0%) and 0.0106 ppm (58.9%) treatment groups was less than 70% of the nominal 0.0032, 0.0058, 0.010, and 0.018 ppm treatment groups, respectively.
7. The 0.058 ppm nominal test concentration was not measured at 96 hours.

Failure to accurately measure the test concentrations that fish were exposed to greatly affected the validity and acceptability of the study.

COMPLIANCE:

Signed and dated GLP, Confidentiality, and Quality Assurance statements were provided. The test was conducted in accordance with the GLP provisions of the "Chemikaliengesetz" (Chemical Act; "Bundesgesetzblatt Jahrgang 1994, Teil I, 29.07.1994") and the OECD Principles of Good Laboratory Practice (Paris, 1981).

A. MATERIALS:

1. Test Material

BAS 500 00 F (Headline Fungicide)

Description:

Brown liquid

Lot No./Batch No. :

97-2

Purity:

247.83 g/L a.i.

Stability of Compound

Under Test Conditions: The stability of the test substance in the dilution water during the course of the study was verified by analytical determination at 0 (96.6-109.4% of nominal) and 96 hours (58.9-80.9%*; Table 9.4, p. 24).

* The 96 hour mean measured concentration was not reported for the 0.058 ppm nominal test concentration.

OECD requires water solubility, stability in water and light, pK_a , P_{ow} , and vapor pressure of the test compound. All OECD requirements were not reported.

Storage conditions of test chemical:

Stored at room temperature.

Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 45826704

2. Test organism:

Species: Bluegill sunfish (*Lepomis macrochirus* RAF.)

Age at test initiation: Not reported

Weight at test initiation: 3.79 g (2.7-5.1 g)

Length at test initiation: 7.03 cm (6.1-7.9 cm)

Source: Osage catfisheries Inc., Osage Beach, Missouri

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study: The definitive nominal test concentrations were based on an acute toxicity test on rainbow trout (LC₅₀ after 96 hours: between 0.0215 and 0.0316 ppm). The nominal concentrations were spaced by a factor of approximately 1.8 and include: 0, 0.0032, 0.0058, 0.010, 0.018, 0.032 and 0.058 ppm "based on the active ingredient", p. 15 of the study report.

b) Definitive Study:

Table 1 . Experimental Parameters

Parameter	Details	Remarks
		Criteria
Acclimation period:	Approximately 21 months.	After arrival, fish were treated "prophylactically" 2 times with 0.05 ppm malachite green chloride and once with 10 ppm tetracycline hydrochloride. EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.
Conditions: (same as test or not)	Fish were kept in a flow-through tank in test dilution water.	
Feeding:	"Tetra Min" standard feed for aquarium fish TETRA - Werke, MELLE, FRG, <i>ad libitum</i> ; and generally on workdays additional frozen and/or live brine shrimp (artemia). Food withdrawn 1 day prior and during exposure	
Health: (any mortality observed)	0.5% mortality during the last 7 days of acclimation.	
Duration of the test	96 hours	EPA/OECD requires: 96 hours

Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 45826704

Parameter	Details	Remarks
		Criteria
<p>Test condition</p> <p>static/flow through</p> <p>Type of dilution system- for flow through method.</p> <p>Renewal rate for static renewal</p>	<p>Static</p> <p>N/A</p> <p>N/A</p>	<p>EPA: Must provide reproducible supply of toxicant, with a consistent flow rate of 5-10 vol/24 hours, and meter systems calibrated before study and checked twice daily during test period</p>
Aeration, if any	The dilution water was aerated prior to testing. No aeration during testing.	<p>EPA requires: no aeration; OECD permits aeration</p>
<p><u>Test vessel</u></p> <p>Material: (glass/stainless steel)</p> <p>Size:</p> <p>Fill volume:</p>	<p>Glass with stainless steel frame</p> <p>80 x 35 x 46 cm</p> <p>100 L (depth of 40 cm)</p>	<p>EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm</p> <p>Fill volume: 15-30 L of solution</p>
Source of dilution water	The dilution water was non-chlorinated, charcoal-filtered and aerated, tap water.	<p>EPA 1975; Soft reconstituted water or water from a natural source, not dechlorinated tap water; OECD permits dechlorinated tap water.</p>

Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 45826704

Parameter	Details	Remarks
		Criteria
<u>Water parameters:</u>		
Hardness	250 mg CaCO ₃ /L	The hardness (250 mg CaCO ₃ /L) was five times higher than the recommended (40-48 mg/L as CaCO ₃). The pH range (8.2-8.5) was greater than recommended (7.2-7.6).
pH	8.2-8.5	
Dissolved oxygen	6.1-8.6 mg/L (≥60% saturation)	
Total Organic Carbon	Not reported	
Particulate Matter	Not reported	The test water was regularly assayed for chemical contaminants and microbes, the Ca content was ± 90 mg/L.
Metals	Mg ± 10 mg/L	
Pesticides	Not reported	
Chlorine	Not reported	
Temperature	23-24°C (continuous measurements: 23.9-25.8°C)	<p>Hardness and pH EPA requires hardness of 40-48 mg/L as CaCO₃ and pH of 7.2-7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes; monthly range <0.8. OECD allows hardness of 10-250 mg/L as CaCO₃ and pH between 6 and 8.5.</p> <p>Dissolved Oxygen <u>Renewal:</u> ≥60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs <u>Flow-through:</u> ≥60% through out test. OECD requires at least 80% saturation value.</p> <p>Temperature EPA requires 22 ± 1 °C for estuarine/marine. OECD requires range of 21 - 25 °C for bluegill and 13-17 °C for rainbow trout.</p> <p>Salinity 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰</p> <p>EPA water quality measured at beginning of test and every 48 hours</p>
{Salinity for marine or estuarine species}	N/A	
Intervals of water quality measurement	DO, pH, and temperature were determined daily in replicates with surviving fish. Additionally, the temperature was measured hourly in one test aquaria (control).	

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish
(*Lepomis macrochirus*)**

PMRA Submission Number {.....}

EPA MRID Number 45826704

Parameter	Details	Remarks
		Criteria
<u>Concentration of test material:</u> nominal: measured:	0 (negative control), 0.0032, 0.0058, 0.010, 0.018, 0.032 and 0.058 ppm. 0, 0.0035, 0.0056, 0.0097, 0.0186, 0.0338 and 0.0609 ppm.	The measured concentrations are the 1-hour mean measured concentrations. The 96-hour measured concentrations were 58.9-80.9% of nominal for the 0.0032, 0.0058, 0.010, 0.018, and 0.032 ppm treatment groups. Due to a lack of a 96-hour measured concentration at the maximum dose level (0.058 ppm nominal), the reviewer was unable to calculate a mean measured value. <i>EPA/OECD requires: Control and five treatment levels. Each conc. should be 60% of the next highest conc., and should be in a geometric series</i>
Solvent (type, percentage, if used)	N/A	<i>EPA requires: Not to exceed 0.5 mL/L for static tests or 0.1 mL/L for flow-through tests; OECD requires solvent, exceed 100 mg/L.</i>
<u>Number of fish/replicates:</u> negative control: solvent control: treated:	10 fish, in one replicate N/A 10 fish, in one replicate	<i>EPA: ≥ 10/concentration; OECD requires at least 7 fish/concentration</i>
Biomass loading rate	0.4 g fish/L	<i>Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through</i>
Lighting	16-hours light/8-hours dark.	<i>EPA requires: 16 hours light/8 hours dark; OECD requires 12 -16 hours photoperiod.</i>

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish
(*Lepomis macrochirus*)**

PMRA Submission Number {.....}

EPA MRID Number 45826704

Parameter	Details	Remarks
		Criteria
Feeding	Animals were not fed during testing.	<i>EPA/OECD requires: No feeding during the study</i>
Recovery of chemical	96.6-109.4% of nominal at 1-hour	Based on analytical recoveries from the 96 hour samples. The 96 hour recoveries for the 0.0032, 0.0058, 0.010, and 0.018 ppm treatment groups were 58.9-65.6% of nominal. These recoveries were less than the recommended 70% of nominal. A 96-hour mean measured concentration was not reported for the 0.058 ppm nominal treatment level.
Level of Quantitation	0.0025 mg/L	
Level of Detection	Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects	
Observation intervals	at 1, 4, 24, 48, 72, 96 hours	<i>EPA/OECD requires: minimally every 24 hours</i>
Were raw data included?	Yes	
Other observations, if any	N/A	

**Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish
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PMRA Submission Number {.....}

EPA MRID Number 45826704

II. RESULTS AND DISCUSSION:

A. MORTALITY:

After 96 hours of exposure, there was 10, 100, and 100% mortality in the 0.0186, 0.0338, and 0.0609 ppm nominal treatment groups, respectively (Table 9.1, p. 21). No mortality was observed in the control, 0.0035, 0.0056, or 0.0097 ppm treatment groups.

Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish
(*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 45826704

Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 45826704

Table 3: Effect of BAS 500 00F (Headline Fungicide) on mortality of Bluegill sunfish (*Lepomis macrochirus*).

Treatment, ppm, measured ^a and (nominal conc.)	No. of fish at start of study	Observation Period							
		0-4 Hours		24 Hours		48 Hours		72-96 Hours	
		No. Dead	% Mortality	No. Dead	% mortality	No. Dead	% mortality	No. Dead	% mortality
Negative control	10	0	0	0	0	0	0	0	0
0.0035 (0.0032)	10	0	0	0	0	0	0	0	0
0.0056 (0.0058)	10	0	0	0	0	0	0	0	0
0.0097 (0.010)	10	0	0	0	0	0	0	0	0
0.0186 (0.018)	10	0	0	0	0	0	0	1	10
0.0338 (0.032)	10	0	0	5	50	10	100	10	100
0.0609 (0.058)	10	7	70	10	100	10	100	10	100
NOAEC (mortality) ^b	0.0080 ppm								
LC ₅₀ (95% C.I.) ^b	0.0146 ppm at the 5% significance level and 0.0299 ppm at the 1% significance level.								
Positive control, if used mortality: LC ₅₀ :	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

^a The mean measured concentrations are based on the study reported 1-hour results.

^b The NOAEC and LC₅₀ were reported by the study author based upon the 1 plus 96 hour mean measured concentrations (p. 13, 33), i.e. the end of the study.

B. NON-LETHAL TOXICITY ENDPOINTS:

The sub-lethal effects included a narcotic-like state in the 0.0609 ppm (two fish), 0.0338 ppm (one fish) and apathy in the 0.0186 ppm (one fish) 1-hour mean measured treatment groups at 1, 24 and 48 hours, respectively (p. 21 of the study report).

Data Evaluation Report on the acute toxicity of BAS 500 00F (Headline Fungicide) to Bluegill Sunfish (*Lepomis macrochirus*)

PMRA Submission Number {.....}

EPA MRID Number 45826704

Table 4. Sublethal effects of BAS 500 00F (Headline Fungicide) on Bluegill sunfish (*Lepomis macrochirus*).

Treatment, ppm, measured ^c and (nominal conc.)	Observation Period				
	endpoint at 1 Hour	endpoint at 24 Hours	endpoint at 48 Hours	endpoint at 72 Hours	endpoint at 96 Hours
	% affected ^a	% affected ^a	% affected ^a	% affected ^a	% affected ^a
Negative control	AN ^b	AN	AN	AN	AN
0.0035 (0.0032)	AN	AN	AN	AN	AN
0.0056 (0.0058)	AN	AN	AN	AN	AN
0.0097 (0.010)	AN	AN	AN	AN	AN
0.0186 (0.018)	AN	AN	Apathy-10%	Apathy-10%	Apathy-10%
0.0338 (0.032)	AN	Narcotic-like state-10%	--	--	--
0.0609 (0.058)	Narcotic-like state-20%	--	--	--	--
NOAEC (sub-lethal)	0.0080 ppm				
LOAEC (sub-lethal)	0.0146 ppm at the 5% significance level and 0.0299 ppm at the 1% significance level.				
EC ₅₀	Not determined				
Positive control, if used % sublethal effect: EC ₅₀ :	N/A ^e	N/A	N/A	N/A	N/A

^a The % affected was reviewer-calculated based on number affected divided by the number of surviving fish.

-- Not determined due to 100% mortality.

^b AN - Appeared Normal

^c The mean measured concentrations are based on the study reported 1-hour results.

^d The NOAEC and LC₅₀ were reported by the study author based upon the 1 plus 96 hour mean measured concentrations (p. 13, 33), i.e. the end of the study.

^e N/A - Not Applicable

C. REPORTED STATISTICS:

The 96-hour LC₅₀ value was estimated using probit analysis at the 1 and 5% significance levels (p. 20 of the study report). The NOAEC was determined based on mortality and sub-lethal effects data. The LC₅₀, NOAEC and LOAEC results were based on the 1 plus 96-hour mean measured concentrations (p. 13, 33), i.e. the mean values calculated from the analytically detected concentrations reported at 1 hour and 96 hours.